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THE EFFECTS OF ANTISERUM ON ADSORBED PHAGE

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THE EFFECTS OF ANTISERUM ON ADSORBED PHAGE

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Harvard Reiter

Medical Bacteriology Division
DIRECTOR OF BIOLOGICAL RESEARCH

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ABSTRACT

The penetration of T3 bacteriophages that are attached to the host cells may be delayed or entirely prevented by antiserum against T3 phage. The antiserum-sensitive period of the attached phages increases at lower temperatures. The degree of inhibition increases with antiserum concentration and with early exposure to antiserum. Bacteriophages T1 and T4 are not affected by antiserum in this manner; T7 reacts similarly to T3.

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I. INTRODUCTION

Phage antiserum modifies free phage so as to inhibit or prevent entirely to normal penetration into the host bacterium.[1,2] Lanni and Lanni[3] demonstrated the presence of two kinds of antibodies in phage antiserum, anti-head and anti-tail. They showed that only anti-tail antibody had a neutralizing effect on phage. From this observation as well as from the electron micrographs showing phage attached to bacteria by the tail[4] they concluded that neutralization was probably due to a physical interference of the antibody between the phage tail and the host receptor sites. This was consistent with experiments of Delbrück, which showed that phage antiserum had no effect on phage replication once the processes of infection were under way. However, Adams and Wassermann[5] demonstrated an inhibitory effect of antiserum on the penetration of T3 that had adsorbed to the host just prior to exposure to antibody. The serum-sensitive period of adsorbed T3 was shown to be less than four minutes at 37°C.

It is the purpose of this paper to investigate the kinetics of the interaction between adsorbed T3 phage and neutralizing antibody.

II. MATERIALS AND METHODS

The organisms used in this study were the B strain of E. coli and the T phages described by Demerec and Fano.[6] The medium used was nutrient broth (Difco) containing 0.5 per cent of added NaCl. All antisera were collected from rabbits at least 28 days after the beginning of immunization. Two anti-T3 sera were used. Their neutralizing potency was characterized by neutralization constants, k, of 150 min^{-1} and 146 min^{-1} at 25°C where $k = D/T \ln P/P_o$ and P/P_o is the fraction of surviving phage after t minutes of exposure to a dilution D of the serum.

The routine techniques used were described by Adams;[7] special techniques are described below.

A. ONE-STEP GROWTH EXPERIMENTS

The one-step growth experiment of Ellis and Delbrück[8] as described by Adams and Wassermann[5] was used to measure the effects of antiserum on phage penetration of the host bacteria. To a 0.9-ml sample of bacteria in logarithmic growth phase was added a 0.1-ml sample of phage. The multiplicity of infection was always less than one. An adsorption period of one minute was allowed in each experiment, after which the adsorption mixture was treated in one of three ways: (a) the mixture was diluted and plated as a control one-step curve for the experimental temperature, or (b) the adsorption mixture was added directly to antiserum for three minutes and a sample of the resulting mixture was then further diluted past the point of significant antiserum concentration and platings for a one-step curve were made, or (c) the adsorption mixture was diluted to prevent further adsorption, kept at the experimental temperature for a given time, added to antiserum for three minutes, and finally, diluted and plated for the one-step curve. The interval between initiation of adsorption and introduction of antiphage serum will be called for convenience the "preserum period." The antiserum dilutions used were such as to give more than 95 per cent inactivation of free phage in three minutes.

B. THE METHOD OF PROBIT ANALYSIS

The results of these experiments were analysed by the probit method of Finney[9] as applied to the one-step growth curve by Adams and Wassermann.[5] A normal one-step growth curve when plotted as a probit curve gives a straight line. This indicates a normal, random distribution of burst times around a mean latent period. The frequency distribution curve will show a deviation from the normal straight-line curve if there is any factor causing a nonrandom distribution in the lysis times of the infected bacterial population.

III. RESULTS

A. EFFECTS OF LOWERED TEMPERATURE ON THE EXPERIMENTAL SYSTEM

At 37°C, T3 phage is susceptible to serum inhibition for only three minutes after adsorption to the bacterial surface. In order to study the system in greater detail, experiments were carried out to determine the effects of lower temperatures on phage-host interaction.

B. ADSORPTION AT DIFFERENT TEMPERATURES

The rate of adsorption of T3 to the bacterial host remained relatively unchanged as the temperature was lowered from 37° to 15°C. From 37° to 25°C the adsorption was irreversible by dilution. However, approximately 41 per cent of the adsorbed phages were eluted from the bacteria on dilution twenty minutes after adsorption at 15°C.

C. EFFECT OF TEMPERATURE ON THE ONE-STEP GROWTH CURVE

A series of one step growth experiments was carried out at various temperatures between 20° and 37°C. The results of these experiments are listed in Table I and representative probit curves are plotted in Figure 1. The effects of lowered temperatures on the rate of phage production can easily be seen in the changed probit plots. The straight-line nature of the probit is retained at all temperatures, indicating that the delay in lysis times is due to random events in a uniform population.

D. EFFECTS OF ANTISERUM ON THE ONE-STEP GROWTH CURVES AT DIFFERENT TEMPERATURES

Experiments to determine the effect of antiserum at successive intervals after adsorption of phage were performed at 37°, 30°, 25°, and 20°C. These experiments were designed so that after a standard one-minute adsorption period there was a variable period before serum was added. It should be emphasized that the new phages measured by the one-step experiment represent the progeny of those phages that had attached irreversibly to the bacteria before antiserum treatment.

The results of these experiments with antiserum are shown in Table I; a representative family of probit curves of experiments run at 25°C at varying "preserum periods" is shown in Figure 2. It is apparent from the probits shown that there is a marked delay in the lysis times of a large proportion of the population when the antiserum is used after a short incubation period. At 25°C, if the preserum period is less than 15 minutes, there is a delay in the lysis times of the entire population. If the preserum period is 20 minutes or longer, the minimum latent period returns to normal, indicating that a measurable proportion of the population lyses normally. The inhibitory effect

TABLE I. EFFECT OF VARIOUS "PRESERUM PERIODS" ON THE LATENT PERIODS AT VARIOUS TEMPERATURES[a]

Preserum period, minutes at __°C	Minimum latent period, minutes	Mean latent period, minutes	Maximum latent period, minutes
37°C	13		
Control	13	17	20
1	15	40	60
4	14	17	19
30°C			
Control	23	30	40
1	26	40	60
2	24	38	56
3	24	35	47
4	24	29	40
25°C			
Control	34	48	60
1	42	70	94
4	42	66	78
6	42	58	75
8	40	58	80
10	38	58	60
15	38	52	70
20	34	47	64
25	34	47	59
20°C			
Control	80	118	154
40	80	122	170

a. The preserum period is the time allowed between the addition of phage and the addition of antiserum to the culture. In the control experiments no antiserum was added. These are the normal one-step growth curves for the given temperatures. The minimum, mean, and maximum latent periods effectively delineate the one-step curves obtained in each experiment.

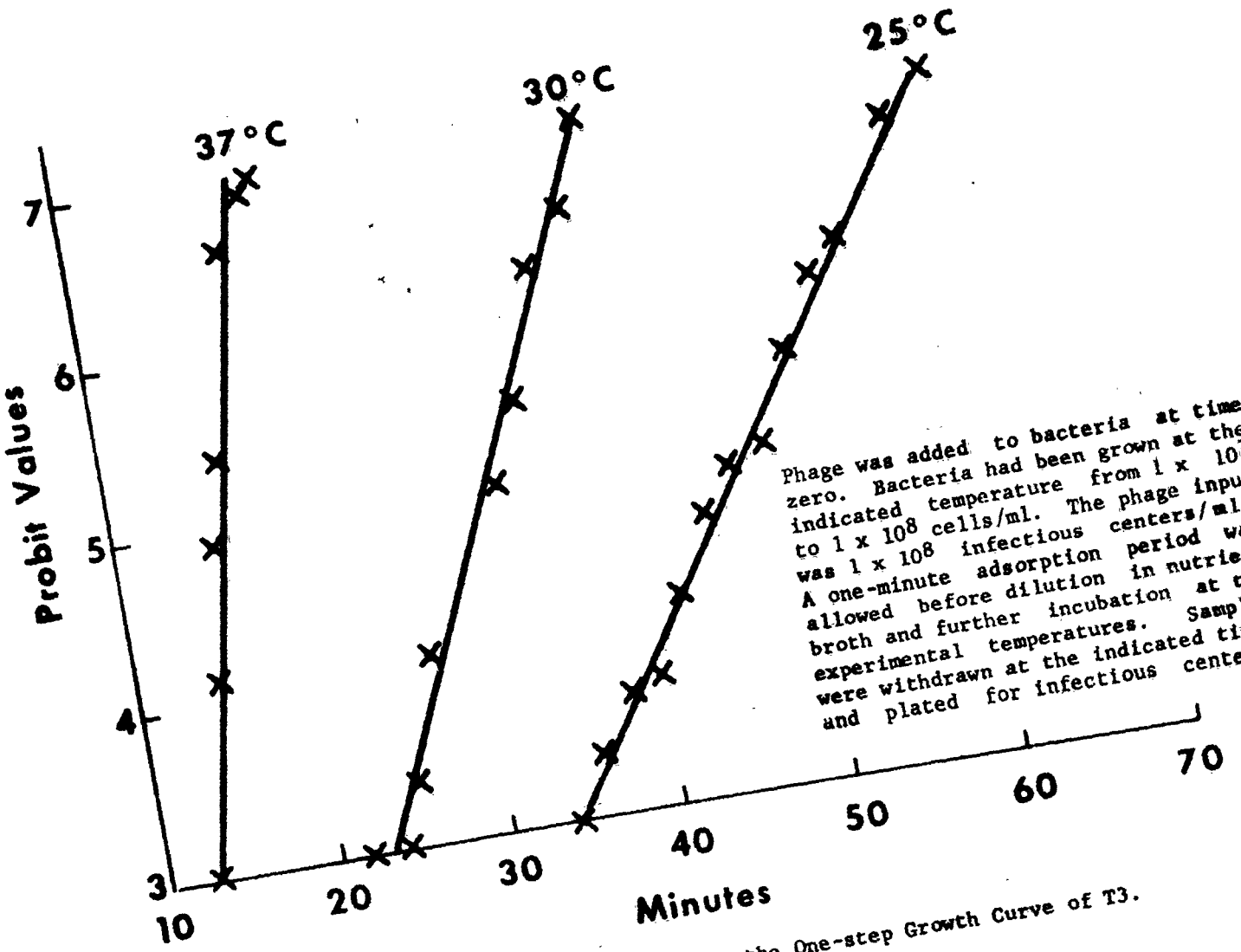

Figure 1. Effect of Temperature on the One-step Growth Curve of T3.

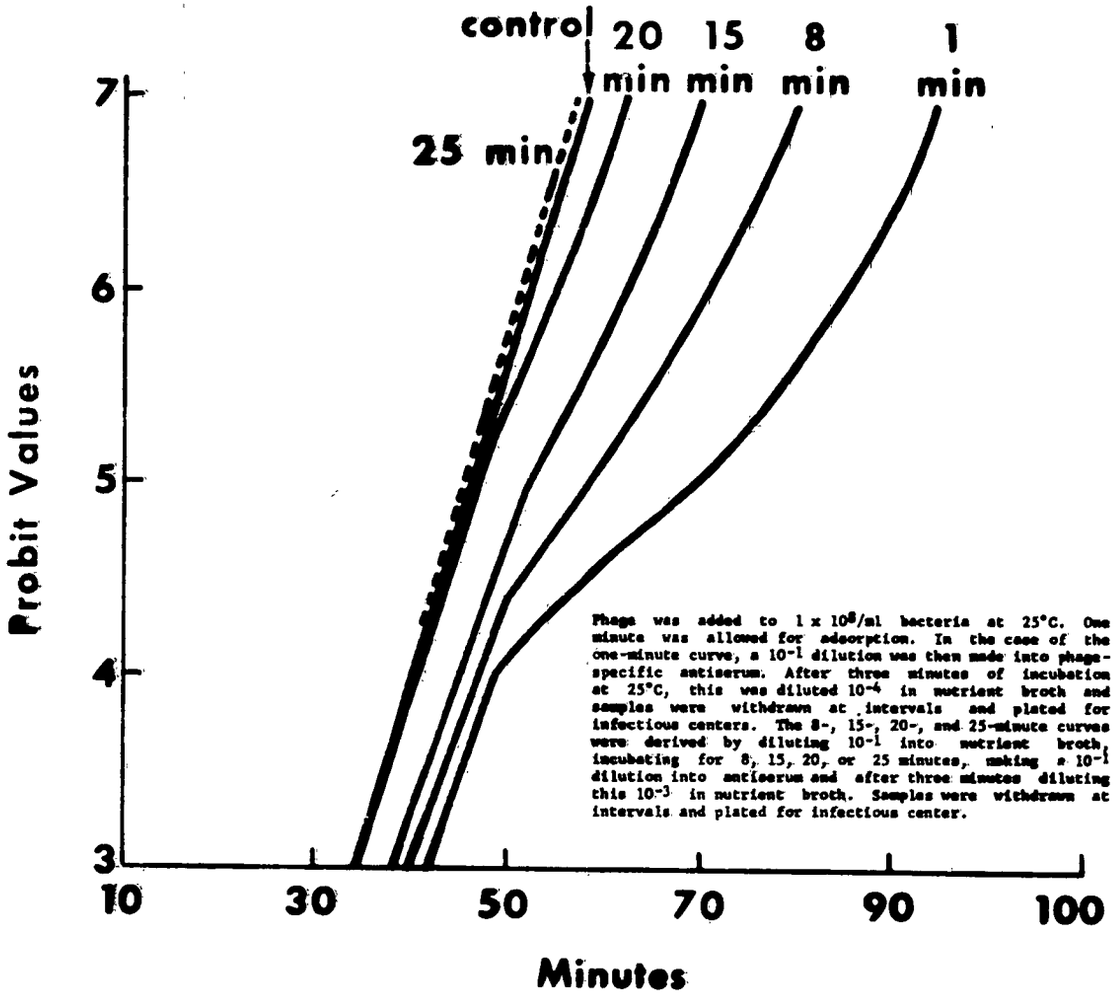

Figure 2. Effect of Preserum Time on the One-step Curve of T3 at 25°C.

of antiserum diminish with increasing preserum periods, although there is a noticeable delay in the lysis of a small segment of the population at 25°C after as long a preserum time as 20 minutes. After a preserum period of 25 minutes at 25°C there is no noticeable effect of antiserum on the population.

The rates at which the mean phage populations escape the inhibitory effects of antiserum at each of a series of different temperatures (Table I) were plotted on an Arrhenius curve. The resulting Arrhenius constant was about 38,160 calories per mole. This suggests that after adsorption and prior to complete penetration there is an antiserum-sensitive stage characterized by a reaction having the Arrhenius constant of 38,160 calories per mole.

E. VARING THE CONCENTRATION OF ANTISERUM

A series of experiments were performed in which varying concentrations of antiserum were used. These experiments were run at 37°C and the standard experimental protocol was followed; the results are shown in Table II. The probit for an experiment using a 1:10 dilution of antiserum rather than the usual 1:100 dilution is shown in Figure 3. It is evident that the higher concentration of antiserum had an increased inhibitory effect on the phage population.

TABLE II. EFFECT OF VARIOUS CONCENTRATIONS OF ANTISERUM ON PHAGE LYSIS TIMES

Preserum period, minutes at 37°C	Antiserum Dilution	Minimum latent period, minutes	Mean latent period, minutes	Maximum latent period, minutes
1	1/400	14	20	34
1	1/100	15	40	60
1	1/10	17	52	88

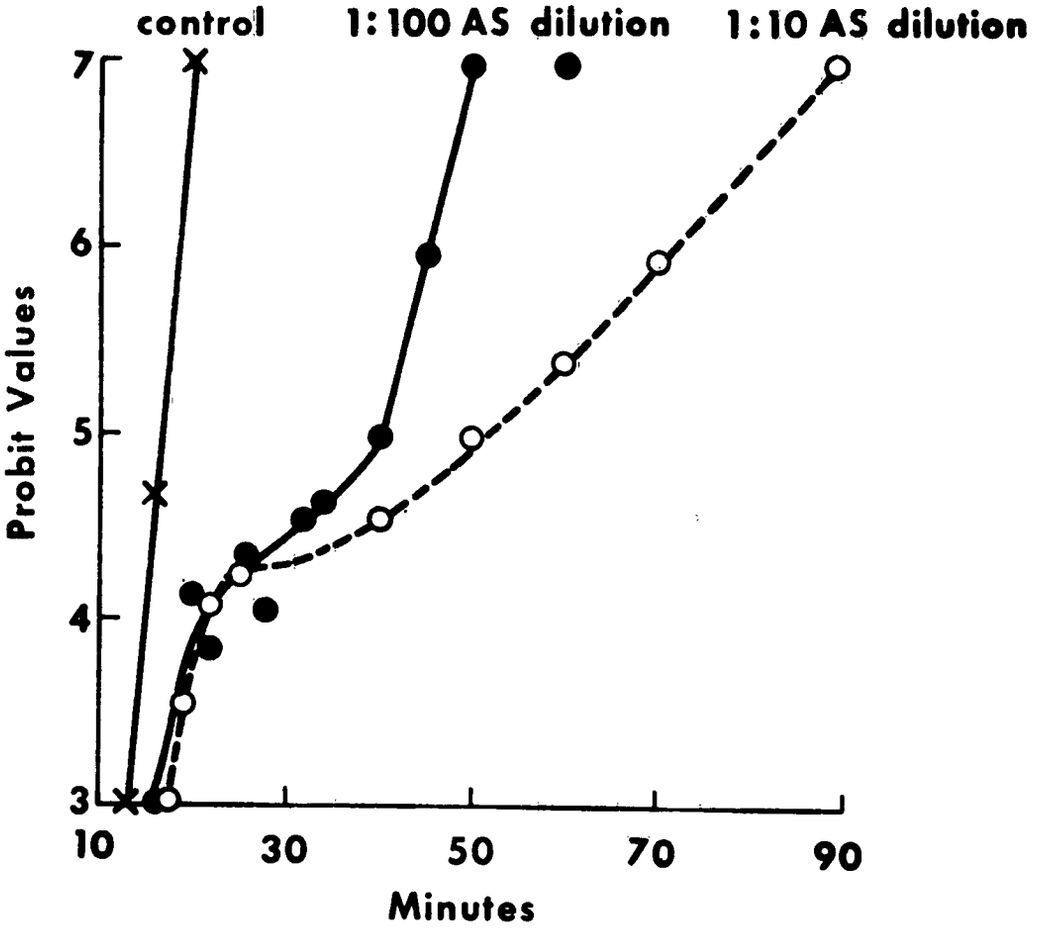

Figure 3. The Effects of 1:100 and 1:10 Antiserum Dilutions on the One-step Growth Curves of T3 at 37°C.

F. COMPLETE NEUTRALIZATION OF ATTACHED T3

It was noted that increasing the strength of the antiserum decreased the number of surviving infectious centers. At 37°C, with 10^8 bacteria per milliliter, about 20 per cent of the phage population adsorbed per minute. If after one minute of adsorption a sample of the mixture was treated with a 1:400 dilution of the T3 antiserum, 11 per cent rather than 20 per cent of the original population survived as infectious centers. Use of a 1:100 antiserum dilution resulted in 4.3 per cent survival, a 1:10 dilution in 2.1 per cent, and a 1:2 dilution gave less than 1.2 per cent survival. Further, the probit plot of the survivors of an exposure to a 1:2 antiserum dilution is a straight line, indistinguishable from the probit plot of a normal, untreated population.

G. PRE-TREATMENT OF PHAGE WITH ANTISERUM

A sample of the free phage stock was treated with a 1:500 dilution of antiserum so that 75 per cent of the phage was inactivated. The 37°C one-step growth curve of the surviving phage showed a four-minute delay in minimum latent period and more than a 20-minute delay in the maximum lysis time. The curve resembled that of the 37°C experiment with a one-minute preserum period.

H. THE EFFECT OF ANTISERUM ON OTHER PHAGES

An attempt was made to determine the effects, if any, of specific phage antiserum of other T phages. The procedures used were the same as those used with the T3 system. A control one-step experiment was run in parallel with the antiserum-treatment experiments. At 37°C, after one minute of adsorption and three minutes of exposure to a 1:10 or a 1:100 dilution of a T4 antiserum ($k = 184\ min^{-1}$), the T4 one-step curve was identical with its control one-step curve.

The same 37°C experiment was used for T1. A 1:50 dilution of a T1 antiserum ($k = 92\ min^{-1}$) produced no deviation from the control probit curve.

Experiments at 37°C with T7, a phage related antigenically to T3, using a 1:100 dilution of a T7 antiserum ($k = 680\ min^{-1}$) gave the curves seen in Figure 4. The delayed lysis of the T7 population resembles closely the similarly treated T3 populations. Approximately 18 per cent of the T7 population was found to be adsorbed after one minute at 37°C, but only 0.1 to 0.2 per cent of the population was recovered as infectious centers when treated with the 1:100 T7 antiserum dilution.

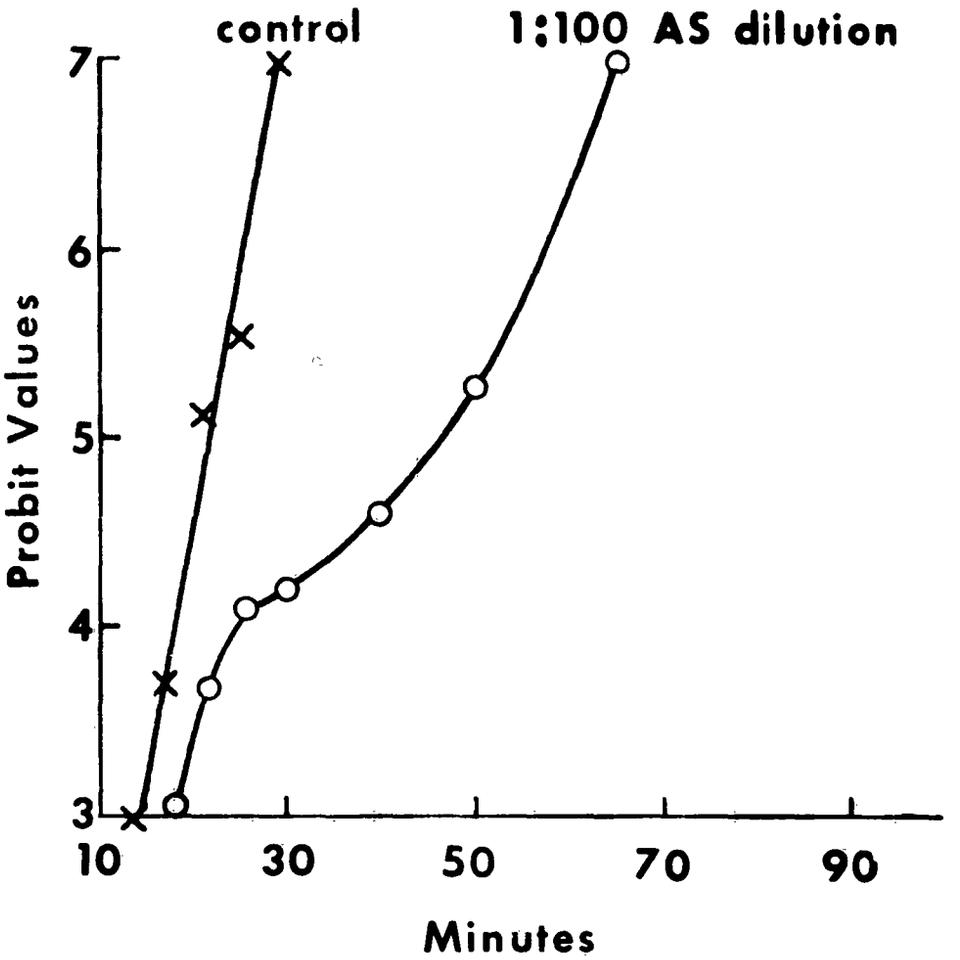

Figure 4. The Effect of T7 Antiserum on the One-step Growth Curve of T7 at 37°C.

I. RECONSTRUCTION AND ANALYSIS OF THE SKEWED PROBIT CURVES

Skewed probit plots similar to those shown in Figure 2 may be obtained by plotting the summation of two normal distribution curves in varying degrees of overlap. However, the reverse process of deriving the distribution curves from the skewed probit requires that certain assumptions be made. These assumptions are (a) that the skewed probit represents a heterogeneous population, and (b) that this heterogeneous population is, for simplicity, made up of only two homogeneous populations.

Figure 5 shows the reconstruction of a probit curve obtained from an experiment with a one-minute preserum period at 25°C. The population of antiserum-inhibited phages has been divided into two equal components. They have straight-line probits, with two different, delayed, mean lysis times.

The calculated curve is derived by dividing the population represented by the normal one-step control curve into two equal portions. These two subpopulations are assigned minimum and maximum latent periods as indicated. They are assumed to lyse normally around a mean and to have the same spread as the control population. The summing of these two subpopulations produces the calculated curve.

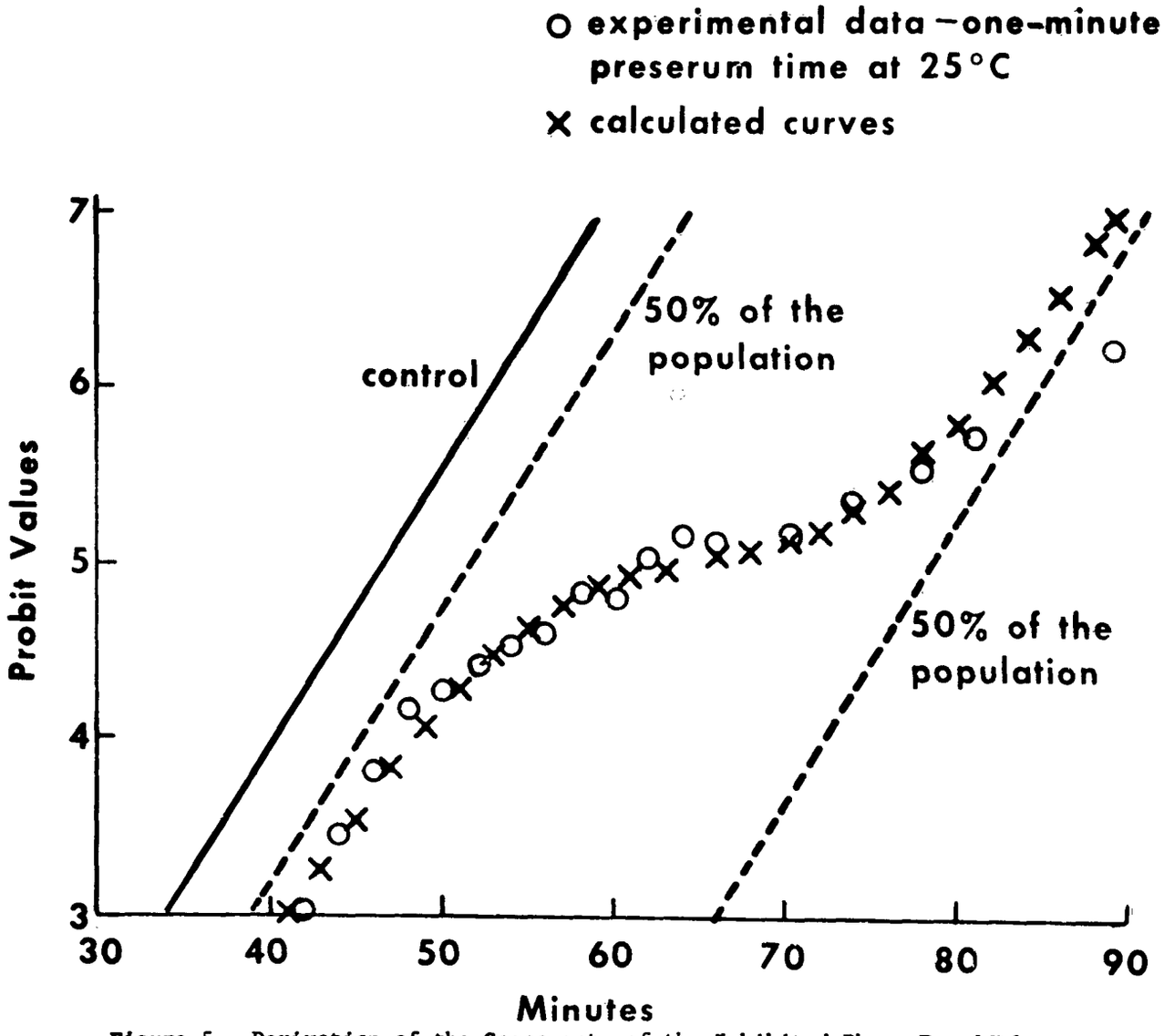

Figure 5. Derivation of the Components of the Inhibited Phage Population.

IV. DISCUSSION

The increased inhibition of the entire population at lowered temperatures or higher antiserum concentrations can not be caused by the increase in the number of antibody molecules reacting at nonspecific or multiple sites on the phage, nor can it be caused by the greater probability of an effective antibody-phage reaction, since either of these two phenomena would result in a random distribution of inhibition states rather than the clear-cut division into two sensitive populations.

A satisfactory explanation of the data must account for the complete inactivation of a portion of the adsorbed phage as well as for the two inhibited populations observed. The one-step growth curve of survivors of a phage population that had been 75 per cent inactivated by antiserum showed inhibition of penetration. Therefore it seems that the inactivation or inhibition of adsorbed phage was due to reactions at different critical phage sites rather than to changes in the phage occurring at different stages in the penetration process itself. If one postulates the existence of two different critical phage sites, A and B, one may expect three different reaction states: when A and B together, A alone, or B alone have reacted with antibody. This hypothesis is consistent with the following scheme of transition from free phage to adsorbed phage, insensitive to antiserum inhibition.

From free phage, the steps of the transition are: (a) adsorbed phage susceptible to complete neutralization, (b) adsorbed phage susceptible to severe inhibition, (c) adsorbed phage susceptible to mild inhibition, and (d) adsorbed or fully penetrated phage insensitive to antiserum inhibition.

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